CHARGE RECOMBINATION KINETICS AS A PROBE OF PROTONATION OF THE PRIMARY ACCEPTOR IN PHOTOSYNTHETIC REACTION CENTERS

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ABSTRACT The kinetics of the charge recombination $D^+Q_A^- \rightarrow DQ_A$ was used to probe the protonation of the primary acceptor in reaction centers from *Rhodopseudomonas sphaeroides*, in which the native ubiquinone was replaced by anthraquinone. We found that Q_A^- is stabilized by the rapid ($t < 10^{-2}$ s) binding of a proton, with a pK of 9.8. The distance between Q_A^- and the proton binding site was estimated to be larger than ~5 Å.

The absorption of light by photosynthetic reaction centers (RCs) leads to a separation of charge between the electron donor, D(a bacteriochlorophyll dimer), and the primary (quinone) acceptor, Q_A, forming $D^+Q_A^-$ (for review, see references 1 and 2). Here we examine the protonation events accompanying the charge separation. Redox titrations of the Q_A/Q_A^- couple (3–5; for review, see reference 6) indicate that Q_A^- associates with a proton ($pK_A = 9.8$). The same conclusion was reached from studies on the kinetics of electron transfer between Q_A^- and the secondary acceptor, Q_B (7). Spectroscopic measurements, however, indicate that the proton is not directly bound to Q_A^-. The optical absorption spectrum of Q_A^- at neutral pH (8–10) resembles that of the unprotonated semiquinone anion (11, 12). Similarly, the EPR spectrum of Q_A^- (13–16) is characteristic of the unprotonated anion (16).

To probe for a protonation site in the vicinity of Q_A, we studied the charge recombination kinetics $D^+Q_A^- \rightarrow DQ_A$, with anthraquinone (AQ) replacing the native ubiquinone (UQ). With AQ, as opposed to UQ, the charge recombination rate, k_{obs}, is sensitive to small perturbations in the free energy of Q_A^- (17, 18). Thus k_{obs} should be sensitive to a shift in the energy of Q_A^- caused by the electrostatic interaction with a nearby proton. A preliminary account of this work has been presented (19).

The charge recombination process of $D^+Q_A^-$ is schematically illustrated in Fig. 1; $k_{H^+}^{on}$ and $k_{H^+}^{off}$ are the rate for proton binding and release, and $k_{obs}^{H^+}$ and k_{obs}^{0} are the recombination rates in the presence and absence of a proton, respectively. The electron on Q_A^- recombines with D^+ via thermal repopulation of the state $D^+I^-Q_A$, where I is the intermediate acceptor (2). The recombination rate depends on the free energy difference, ΔG^0, between $D^+I^-Q_A$ and $D^+IQ_A^-$ (18). The shift in this energy difference, δG^0, caused by the binding of a proton, results in a change in the rate given by (18):

$$k_{obs}^{H^+} = k_{obs}^{0}\, e^{-\delta G^0/k_bT}, \qquad (1)$$

where k_b is Boltzmann's constant and T the absolute temperature.

The time dependence of the recombination kinetics, k_{obs}, will depend on whether the proton equilibration rate, $k_{on}^{H^+} + k_{off}^{H^+}$ (Fig. 1), is fast or slow with respect to the charge recombination rates $k_{obs}^{H^+}$ and k_{obs}^{0}. If the proton equilibration rates is fast, the states[1] $(D^+Q_A^-)H^+$ and $D^+Q_A^-$ are in equilibrium on the time scale of the charge recombination; the observed kinetics will follow a single exponential decay, with k_{obs} given by the sum of $k_{obs}^{H^+}$ and k_{obs}^{0} weighted by the fraction of unprotonated and protonated RCs, respectively, i.e.,

$$k_{obs} = \frac{k_{obs}^{0} + 10^{(pK_A - pH)}\, k_{obs}^{H^+}}{1 + 10^{(pK_A - pH)}}, \qquad (2)$$

where

$$(pK_A - pH) = \log \frac{[(D^+Q_A^-)H^+]}{[D^+Q_A^-]} = \log \frac{k_{on}^{H^+}}{k_{off}^{H^+}}. \qquad (3)$$

When the proton equilibration rate is slow compared with $k_{obs}^{H^+}$ and k_{obs}^{0}, the states $(D^+Q_A^-)H^+$ and $D^+Q_A^-$ do not interconvert on the time scale of the charge recombina-

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[1]These states contain the intermediate state, *I*, which we have omitted for the sake of simplicity.

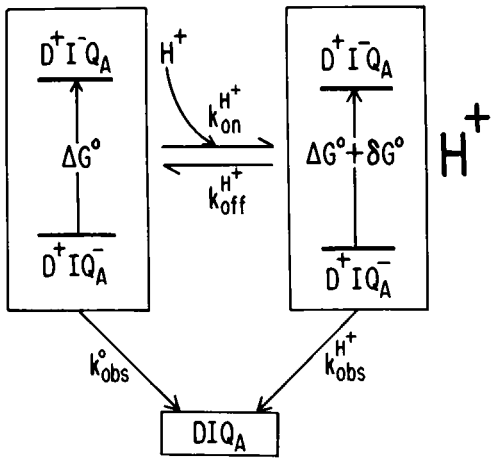

FIGURE 1 Schematic representation of the change in energy levels of D^+I^-Q and $D^+IQ_A^-$ upon protonation. When the primary quinone is anthraquinone, the charge recombination $D^+IQ_A^- \xrightarrow{k_{obs}} DIQ_A$ proceeds via the thermally excited state $D^+I^-Q_A$ and depends, therefore, exponentially on the energy difference $\Delta G^0 + \delta G^0$. (18)

tion, and the observed kinetics will follow two expoential processes; RCs in the state $D^+Q_A^-$ recombine with rate k_{obs}^0, while those in the state $(D^+Q_A^-)H^+$ recombine with rate $k_{obs}^{H^+}$.

The recombination kinetics were measured by monitoring the optical absorption change ΔA^{865}; this change corresponds to the formation and subsequent decay of D^+ (1). Experiments were performed using RCs isolated from *R. sphaeroides* R-26 (1), depleted of the native UQ (20), and reconstituted with either AQ or UQ (18, 20). Flash-induced charge separation was accomplished with a pulsed dye laser (λ_0 = 584 nm, 0.4 μs pulse width, 0.2 J/pulse). Changes in optical absorption were recorded with a spectrophotometer of local design (7). Experimental conditions were as described (7).

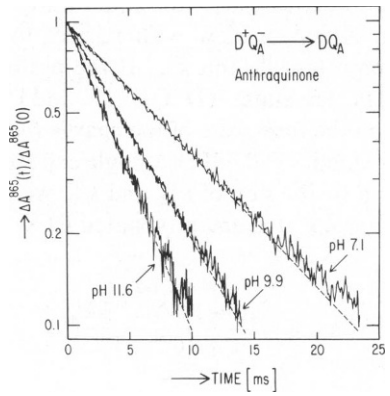

FIGURE 2 Semilogarithmic plot of the change in optical absorption at 865 nm after a single, saturating flash of light (0.4 μs pulse width, 0.2 J/pulse) for different values of pH. The contribution to the kinetics from a residual (~10%) population of RCs containing UQ was measured before the addition of AQ and was subtracted in the data shown. The solid lines represent the best fit of the function $\Delta A^{865}(t)/\Delta A^{865}(0) = e^{-k_{obs}t}$ to the data. The observed kinetics were independent of the light intensity for 10^5 W/cm^2 < I < 10^6 W/cm^2. Conditions: To 1.8 μM RCs depleted of UQ were added, 20 μM AQ in either 10 mM PIPES (piperazine-*N*, *N*'-bis-[2-ethanesulfonic acid]) (pH 7.1), 10 mM CHES (cyclohexylamino-ethanesulfonic acid) (pH 9.9) or 10 mM CAPS [3-(cyclohexylamino)-propanesulfonic acid] (pH 11.6), and 0.025% (wt/vol) LDAO (lauryl dimethylamine-*N*-oxide) at T = 21°C.

The recovery kinetics $D^+ \rightarrow D$ are shown in Fig. 2. The decrease in the recovery rate with decreasing pH implies that $D^+Q_A^-$ is stabilized by the presence of a proton. The kinetics followed a single-exponential decay at all pH values (Fig. 2), implying that the proton binds rapidly compared with k_{obs}, i.e.,

$$k_{on}^{H^+} + k_{off}^{H^+} \gg k_{obs} \sim 10^2 s^{-1}. \quad (4)$$

This limit is consistent with the results of proton uptake measurements (21–23); the stoichiometry of the measured uptake is, however, controversial (21–24).

The pH dependence of k_{obs} is shown in Fig. 3. The value of k_{obs} at low pH is in agreement with previous findings (17, 18, 25). To verify that the increase in k_{obs} at high pH was not an artifact of the quinone removal and reconstitution process, measurements were performed with RCs reconstituted with UQ. The rate k_{obs} changed by <25% over the range 6 < pH < 11, in agreement with results for native RCs (7). The solid line in Fig. 3 represents the best fit of the kinetics model (Eq. 2) to the data with $k_{obs}^{H^+}$ = 97 s^{-1}, k_{obs}^0 = 230 s^{-1} and pK_A = 9.8. Note that the value of pK_A matches that found from both redox titrations (3–5) and electron transfer (7).

The interaction energy of Q_A^- with a nearby proton depends on $k_{obs}^{H^+}$ and k_{obs}^0 (see Eq. 1). The energy splitting δG^0 is given by (Eq. 1 with T = 21°C):

$$\delta G^0 = k_b T \ln \frac{k_{obs}^0}{k_{obs}^{H^+}} = 22 \text{ meV}. \quad (5)$$

The magnitude of δG^0 is approximately two orders of magnitude smaller than the optical transition energy of

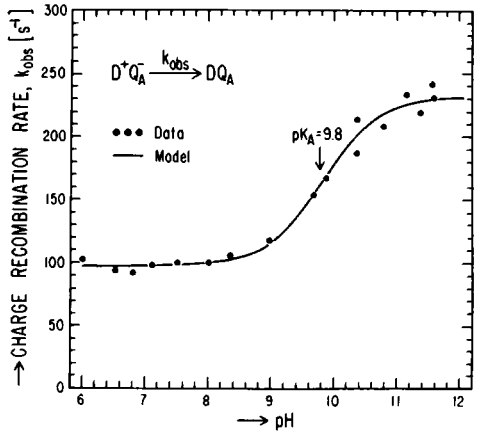

FIGURE 3 The pH dependence of the charge recombination rate k_{obs}. The solid line (Model) was calculated using Eqs. 2 and 3 with pK_A = 9.8, $k_{obs}^{H^+}$ = 97 s^{-1} and k_{obs}^0 = 230 s^{-1}. Conditions as in Fig. 2, except for varying buffers and pH.

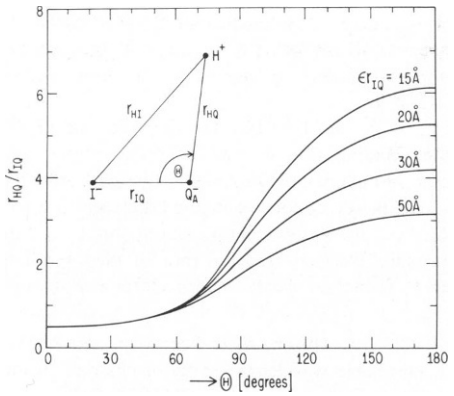

FIGURE 4 The normalized proton to Q_A^- distance, (r_{HQ}/r_{IQ}), as a function of the angle, Θ, between the $I^- - Q_A^-$ and the $H^+ - Q_A^-$ axes, for different values of ϵr_{IQ}. The curves were calculated using Eqs. 5 and 6. Inset shows the geometrical arrangement of the reactants.

Q_A^- (9–11). This may explain why the optical spectrum of Q_A^- is essentially unaffected by the binding of a proton.[2]

From the value of δG^0, one can make a rough estimate of the location of the proton binding site relative to Q_A^-. We assume that the interaction of the proton with both Q_A^- and I^- is electrostatic in origin, as has been done to explain the effects of point charges in other photosynthetic systems (26–29). The change in energy δG^0 is caused by the difference in the distance of the proton to Q_A^- (i.e., r_{HQ}) compared with the distance to I^- (i.e., r_{HI}). From Coulomb's law we obtain[3]

$$\delta G^0 = \frac{-e^2}{\epsilon r_{HI}} - \frac{-e^2}{\epsilon r_{HQ}}$$

$$= \frac{-e^2}{\epsilon r_{IQ}}\left[\frac{1}{[1 + (r_{HQ}/r_{IQ})^2 - 2(r_{HQ}/r_{IQ})\cos\theta]^{1/2}} - \frac{1}{(r_{HQ}/r_{IQ})}\right], \quad (6)$$

where e is the electronic charge, ϵ is the effective dielectric constant, r_{IQ} is the distance between the I^- and Q_A^- and Θ is the angle between the $I^- - Q_A^-$ and $H^+ - Q_A^-$ axes (see insert in Fig. 4). The distance r_{IQ} has been estimated from spectroscopic measurements to be 8–12 Å (30,31). The value of ϵ is difficult to estimate when one is dealing with distances on the scale of atomic dimensions[4] (see references 32–35). Consequently, we have not assumed a specific value of ϵ, but have calculated distances in terms of ϵr_{IQ}. By equating Eqs. 5 and 6, permissible combinations of (r_{HQ}/r_{IQ}) and θ were determined for the range of 15 Å $\leq \epsilon r_{IQ} \leq$ 50 Å (Fig. 4). The minimum distance of the proton to Q_A^- occurs when the proton is situated about halfway between I^- and Q_A^-, i.e., $\theta = 0$. At this location, $r_{HQ} \simeq 0.5\, r_{IQ} \simeq 5$ Å; the ratio r_{HQ}/r_{IQ} is essentially independent of ϵr_{IQ}.

In conclusion, we have shown that a proton is associated with Q_A^- at a distance $\gtrsim 5$ Å. This relatively large distance may explain why the protonation has not been observed in either the optical or EPR spectrum of $(Q_A^-)H^+$. The pK that we determined (9.8) is in agreement with the values found from redox (3–5) and electron transfer (7) studies.

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[2]Although δG^0 represents the change in energy difference between two states (Fig. 1), the shifts in the individual energies are expected to be also small with respect to the optical transition energy.

[3]We assume that the relative distances between the reactants D^+, I^-, Q_A^- do not change upon protonation.

[4]It will be of interest to obtain the value of ϵ when the distances will have been determined from crystallographic and/or electron nuclear double resonance measurements.

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